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# Hydrolytic reaction catalyzed by poly[*N*-(substituted)glycine]s having imidazolyl groups in side chains in the presence of liposome

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Hydrolytic reactions in the presence of liposomes catalyzed by $N^{\epsilon}$-benzyloxycarbonylhistidine groups introduced into the side chains of poly[*N*-(3-aminopropyl)glycine] were studied. On increasing the hydrophobicity of the polypeptide catalyst by introducing dodecyl groups into the side chains, and in the presence of dipalmitoylphosphatidylcholine (DPPC) bilayer membranes, *p*-nitrophenyl palmitate (PNPP) was hydrolyzed more rapidly than *p*-nitrophenyl acetate (PNPA). The addition of cholesterol or phosphatidylserine to lipid bilayer membranes accelerated the hydrolysis of PNPP catalyzed by the polypeptide catalyst more strongly than that of PNPA. The substrate selectivity and catalytic efficiency of the polypeptide catalyst were found to be controlled by the physical state of the lipid bilayer membranes.

## 1. Introduction

The significance of membrane-bound enzymes in cell physiology is well recognized. For example, in signal transduction through cell membranes, membrane-bound enzymes play important roles. Phospholipase C breaks down phosphatidylinositol [1], and protein kinase C phosphorylates proteins [2]. Adenylate cyclase regulates the cAMP level in the cytosol [3].

Generally, the activities of membrane-bound enzymes are enhanced enormously upon binding to lipid membranes. For example, on incorporation of adenylate cyclase into liposomes, the activity increases up to 100-fold of that in the absence of liposomes [4]. Therefore, the activities of membrane-bound enzymes must be closely related to the nature of the membrane environment [5]. In the present investigation, we aimed at elucidating the fundamental aspects of the enzymatic activity of amphiphilic polypeptides in the presence of lipid membranes as models for membrane-bound enzymes.

A number of polymer models for hydrolytic enzymes have been constructed and investigated in aqueous solution. Kiefer et al. [6] have synthesized branched poly(ethyleneimine)s containing nucleophilic catalytic and hydrophobic binding groups, and found the polymers to be effective in hydrolysis of hydrophobic esters. Overberger and Salamone [7] have reported efficient solvolytic activities of poly(vinylimidazole)s and copolymers. On the other hand, Kunitake and Shinkai [8] were interested in the charge relay system found in serine proteases, and synthesized vinyl polymers carrying imidazole, phenol, and carboxylate groups as functional groups. Murakami et al. [9] investigated the catalytic activity of micellar surfactants containing a histidyl residue. However, these polymer catalysts have not been studied with respect to catalysis in lipid bilayer membrane systems.

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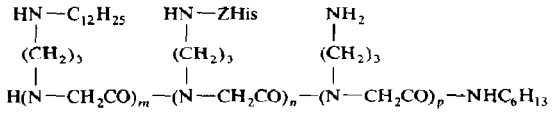

$m = 0,\ n = 4,\ p = 31$: poly(ZHis$_4$-NAPGly) (1)

$m = 1,\ n = 3,\ p = 31$: poly($C_{12}$ZHis$_3$-NAPGly) (2)

ZHis-NHC$_6$H$_{13}$: ZHis-hexylamide (3)

Scheme 1. Structure of poly[*N*-(substituted)glycine].

Since amphiphilic compounds tend to associate with lipid membranes [10], they might be suitable as models of membrane-bound enzymes. We have recently reported strong interactions for poly[*N*-(3-aminopropyl)glycine], poly(NAPGly), and its derivatives with lipid membranes [11]. These polymers are characterized by having a flexible main chain as a result of the *N*-substitution of amide linkages, which leads to elimination of hydrogen bonding by amide protons and *cis / trans* isomerization around the *N*-substituted amide bonds [12]. $N^{\epsilon}$-Benzyloxycarbonylhistidyl (ZHis) and dodecyl ($C_{12}$) groups were introduced into the polymer side chains as nucleophilic catalytic sites and anchors to the lipid membrane, respectively (scheme 1). Such water-soluble polymers were found to bind effectively to lipid bilayer membranes without disrupting the membrane structure. In the present article, the hydrolysis of carboxylic acid esters catalyzed by these polymers in the presence of lipid bilayer membranes was investigated.

## 2. Experimental

### 2.1. Materials

*p*-Nitrophenyl acetate (PNPA; from Wako, Japan) was recrystallized from ethanol. *p*-Nitrophenyl palmitate (PNPP) was synthesized by condensation of palmitic acid (1.0 g) and *p*-nitrophenol (651 mg) in chloroform using dicyclohexylcarbondiimide (DCCD, 885 mg) as coupling reagent, and recrystallized from ethanol [13]; yield 825 mg (56%), m.p. 61° C. (Found: C, 69.84; H, 9.50; N, 3.47%. Calc. for $C_{22}H_{35}NO_4$: C, 69.99; H, 9.34; N, 3.71%).

The structure of the catalysts is shown in scheme 1. They were synthesized as reported previously [11].

$N^{\epsilon}$-Benzyloxycarbonylhistidine *n*-hexylamide (ZHis-hexylamide [3]). $N^{\alpha,im}$-Bis(benzyloxycarbonyl)histidine (5.0 g) was reacted with *p*-nitrophenol (1.9 g) in chloroform using DCCD (2.4 g) [14]. The resulting active ester (4.8 g) was dissolved in chloroform, and reacted with *n*-hexylamine (1.8 ml) at room temperature. The reaction mixture was stirred overnight, followed by evaporation of the solvent. The residue was dissolved in ethyl acetate, then washed with water, 1 N $NH_4OH$, and water. The ethyl acetate solution was dried over $Na_2SO_4$, evaporation of the solvent being subsequently carried out. The oil obtained was purified by chromatography on a silica gel column using chloroform as eluant, and on an LH20 column employing methanol as eluant; yield 953 mg (29% from the ester), m.p. 168–170° C. (Found: C, 64.25; H, 7.95; N, 15.23%. Calc. for $C_{20}H_{28}O_3N_4$: C, 64.49; H, 7.58; N, 15.04%).

Commercial imidazole (obtained from Wako) was recrystallized from benzene.

### 2.2. Preparation of vesicles

Commercial dipalmitoylphosphatidylcholine (DPPC, Sigma), dimyristoylphosphatidylcholine (DMPC, Sigma), phosphatidylserine (PS, Sigma) and cholesterol (Chol, Wako) were used without further purification. Lipid dispersions in a buffer solution containing Hepes (10 mM), NaCl (0.1 M) and EDTA (0.1 mM) were sonicated at pH 7.8 under an atmosphere of $N_2$ above the phase-transition temperature ($T_c$) of the lipid membrane, and ultracentrifuged at $100\,000 \times g$.

Lipid concentration was determined according to the method reported by Raheja et al. [15].

### 2.3. Measurements

Fluorescence and absorption spectra were recorded on a Hitachi MPF-4 fluorescence spectrophotometer and a Jasco UVIDEC-1 spectrometer, respectively. A cell having an optical path length of 5 cm was used for the ultraviolet measurements.

Fluorescence depolarization was measured on a modified MPF-4 apparatus as reported previously [11]. The excitation wavelengths for 1,6-diphenyl-1,3,5-hexatriene (DPH), 2-(9-anthroyloxy)palmitic acid (2AP) and 16-(9-anthroyloxy)palmitic acid (16AP) were 360, 365 and 365 nm, respectively [16].

### 2.4. Distribution of substrate and catalyst binding to lipid membranes

The distribution of a substrate or catalyst bound to a lipid membrane was determined by using a column or centrifugation method. In the former, liposomes (10 mM) were incubated with substrate ($2 \times 10^{-5}$ M) and substrate in solution was separated from that in liposomes by elution through a Sepharose 4B column using Hepes buffer as eluant. To each fraction were added Triton X-100 to break down the liposomes and NaOH to hydrolyze the substrate, the substrate concentration being subsequently determined by measuring the absorbance at 400 nm, due to picrate anions liberated from the substrate. In the centrifugation method, multilamellar vesicles (MLVs) were prepared by weak sonication of lipid dispersions using a bath-type sonicator. MLVs (2.9 mM) were incubated with catalyst ($8 \times 10^{-5}$ M) at 45 °C for 10 min. The suspension was centrifuged at 10000 $\times g$ for 20 min, with the concentration of catalyst in the supernatant being determined by a fluorescence method using fluorescamine.

### 2.5. Hydrolysis of carboxylic acid esters

The hydrolysis of *p*-nitrophenyl acyl derivatives was carried out at pH 7.8 (Hepes buffer), at 35 °C (below $T_c$) or 45 °C (above $T_c$). The hydrolytic reaction in a 50% dioxane/50% buffer mixture was carried out at $3 \times 10^{-4}$ M imidazolyl and $3 \times 10^{-5}$ M substrate. In contrast, hydrolysis in the presence of 1 mM liposomes was performed with imidazolyl and substrate at $2 \times 10^{-5}$ and $2 \times 10^{-6}$ M, respectively.

The pseudo-first-order rate constant, $k_1$, was determined from a first-order plot of the absorbance at 400 nm vs. time. $k_1$ was corrected with respect to the spontaneous hydrolysis rate constant ($k_w$), and divided by the apparent imidazolyl concentration in the lipid membranes ([E]) to give the second-order rate constant ($k_{cat}$) [13].

$$k_1 t = \ln(A_\infty - A_0)/(A_\infty - A_t)$$

$$k_{cat} = (k_1 - k_w)/[E]$$

The first-order plot for hydrolysis of PNPP or PNPA by ZHis-hexylamide (**3**) in the presence of DPPC liposomes at 35 and 45 °C was linear up to nearly 90% conversion, at which the concentration of liposomes was in the range 0.5–1.0 mM.

## 3. Results and discussion

### 3.1. Distribution of substrate and polymer catalyst to liposomes

The distributions of the respective fractions of substrates and catalysts to DPPC liposomes at 45 °C were determined to be 78% for PNPP (column method), 54% for poly(ZHis$_4$-NAPGly) (**1**), 58% for poly($C_{12}$ZHis$_3$-NAPGly) (**2**) and 75% for ZHis-hexylamide (**3**) (centrifugation method). In contrast, only a small amount of PNPA was found to be bound with the lipid membrane.

In order to obtain further information regarding the penetration of substrates and catalysts into membranes, the changes in membrane fluidity induced by addition of substrates or catalysts were investigated by monitoring the fluorescence depolarization of DPH. The experimental value is related to the membrane fluidity of the hydrophobic core of lipid bilayer membranes. The change in fluorescence polarization of DPH induced by the additives relative to that in their absence was evaluated, thus yielding values of the relative change in fluorescence polarization, as shown in table 1. The positive values listed in table 1 signify that the additives induced a decrease in membrane fluidity.

PNPA did not result in any marked alterations in membrane fluidity. On the other hand, the hydrophobic substrate, PNPP, strongly decreased the membrane fluidity. The results indicate that PNPP is taken up into the hydrophobic core of

Table 1

Relative change in fluorescence polarization of DPH in DPPC liposomes induced by addition of various substrates or catalysts

Relative change in fluorescence polarization (%) is defined as $100\times(P-P_0)/P_0$, where $P$ represents the average fluorescence polarization 5 min after the addition of substrate or catalyst (0.02 mM), $P_0$ denoting fluorescence polarization in the absence of additives.

| | Relative change in fluorescence polarization (%) | |
|---|---|---|
| | 35 °C | 45 °C |
| PNPA | 0.03 | 0.8 |
| PNPP | 2.5 | 3.2 |
| Poly($ZHis_4$-NAPGly) (1) | 0.3 | 0.2 |
| Poly($C_{12}ZHis_3$-NAPGly) (2) | 1.8 | 2.7 |
| ZHis-hexylamide (3) | 2.7 | 3.7 |

lipid bilayer membranes, while binding, if any, of PNPA to the membrane surface is only weak.

Poly($ZHis_4$-NAPGly) (1) has been reported to become adsorbed only to the membrane surface [11], in agreement with the small change in membrane fluidity observed in the present investigation. Although the fraction of poly($C_{12}ZHis_3$-NAPGly) (2) bound to membranes was similar to that of poly($ZHis_4$-NAPGly), poly($C_{12}ZHis_3$-NAPGly) (2) gave rise to significant changes in membrane fluidity, indicating that this polymer should be tightly bound to the lipid membrane. On the other hand, ZHis-hexylamide (3), a reference compound of low molecular weight, showed the most prominent effect in decreasing the membrane fluidity, indicating that it takes up position in the hydrophobic core of lipid bilayer membranes.

### *3.2. Interaction of polymer catalyst, with various kinds of liposomes*

Cholesterol or the negatively charged lipid, PS, was added to DPPC liposomes, and the effect of various lipids on the interaction of polymer catalysts with lipid bilayer membranes was ascertained by monitoring the fluorescence depolarization of 2AP, 16AP or DPH. The fluorescent probe, 2AP, provides a measure of the fluidity of the membrane surface, while 16AP and DPH yield such data in the case of the hydrophobic core of lipid bilayer membranes. The experimental data are summarized in table 2.

Table 2

Relative change in fluorescence polarization induced by addition of polymer catalysts

Values were calculated in the same manner as described in table 1.

| Temperature (°C) | Liposome composition | Probe | Relative change of fluorescence polarization (%) | |
|---|---|---|---|---|
| | | | Poly($ZHis_4$-NAPGly) (1) | Poly($C_{12}ZHis_3$-NAPGly) (2) |
| 35 | DPPC | 2AP | 3.9 | 1.5 |
| 35 | 20% Chol/DPPC | 2AP | 7.2 | – |
| 35 | DPPC | 16AP | 2.6 | 16.7 |
| 35 | 20% Chol/DPPC | 16AP | 5.5 | – |
| 35 | DPPC | DPH | 0.3 | 1.8 |
| 35 | 20% Chol/DPPC | DPH | 0.5 | 4.2 |
| 45 | DPPC | 2AP | 2.4 | 6.4 |
| 45 | 20% Chol/DPPC | 2AP | 3.6 | – |
| 45 | 1% PS/DPPC | 2AP | 3.0 | – |
| 45 | DPPC | 16AP | 1.1 | 10.4 |
| 45 | 20% Chol/DPPC | 16AP | 9.6 | – |
| 45 | 1% PS/DPPC | 16AP | 4.7 | – |
| 45 | DPPC | DPH | 0.2 | 2.7 |
| 45 | 20% Chol/DPPC | DPH | 0.3 | 5.4 |
| 45 | 1% PS/DPPC | DPH | 0.3 | 2.3 |

Addition of cholesterol to DPPC membranes increased the relative change in fluorescence polarization induced by polymer addition. Binding of polymer catalysts to lipid membranes should be enhanced by the presence of cholesterol in the membranes. This tendency was observed either above or below the phase-transition temperature. Furthermore, membrane fluidity at the membrane surface, assessed by using 2AP, as well as that in the hydrophobic core of lipid bilayer membranes, as determined by employing 16AP, was found to decrease. Cholesterol is known to reduce membrane fluidity above the phase-transition temperature, and to loosen the packing of lipid molecules below it [17]. Epand et al. [18] reported that an amphiphilic polypeptide, glucagon, readily underwent binding to lipid membranes in the gel state via formation of a highly ordered complex of glucagon with lipid molecules. One might therefore suggest that binding of an amphiphilic polypeptide to lipid membranes is enhanced by a reduction in mobility of lipid molecules above $T_c$, whereas, below $T_c$, the molecules of lipid are packed too closely for incorporation of the polypeptide to occur. Addition of cholesterol to the gel-state membrane would facilitate binding of the polypeptide to the lipid membranes.

On conferring a negative charge on the surface of DPPC membranes by adding PS, the relative change in fluorescence depolarization of the membrane induced by polymer addition was more extensive as compared to the absence of PS. Since the polypeptide is positively charged, it must become tightly bound to the lipid membrane due to electrostatic attraction.

### 3.3. Hydrolysis of p-nitrophenyl esters by polymer catalysts in the presence of liposomes

The degree of binding of PNPA to lipid membranes was estimated to be low, however, hydrolysis of PNPA by poly(ZHis$_4$-NAPGly) (**1**) in the presence of DPPC liposomes ($k_{cat} = 151$ $M^{-1}$ $min^{-1}$) was 5-fold faster than that in buffer solution (28.4 $M^{-1}$ $min^{-1}$) at 55 °C (above $T_c$). We ascribe the enhancement in the rate of reaction of PNPA in the presence of liposomes as being due to weak condensation of PNPA and the polymer catalyst at the membrane surface. In this respect, hydrolysis of PNPA at the membrane surface and of PNPP in the hydrophobic core of the lipid bilayer membrane can be discussed in terms of differences in the extent of polypeptide catalyst-membrane interactions.

The experimental results on the hydrolytic reaction above $T_c$ are summarized in table 3. The values listed are of the relative rate constant ($k_{cat}^{rel}$), $k_{cat}$ for hydrolysis by imidazole in a 50% dioxane/50% buffer mixture being taken as standard.

Table 3

Relative rate constant for hydrolysis of *p*-nitrophenyl esters at 45 °C in the presence of liposomes

Values of $k_{cat}$ (in l $mol^{-1}$ $min^{-1}$) for the hydrolysis of PNPA and PNPP by imidazole in 50% dioxane/50% buffer mixture were 6.7 and 2.9, respectively.

| Catalyst | Solvent | $k_{cat}^{rel}$ | |
|---|---|---|---|
| | | PNPA | PNPP |
| Imidazole | 50% dioxane | 1.0 | 1.0 |
| ZHis-hexylamide (**3**) | 50% dioxane | 0.39 | 0.38 |
| Poly(ZHis$_4$-NAPGly) (**1**) | DPPC liposomes | 11.7 | 2.0 |
| Poly(C$_{12}$ZHis$_3$-NAPGly) (**2**) | DPPC liposomes | 27.3 | 20.6 |
| ZHis-hexylamide (**3**) | DPPC liposomes | 13.4 | 194 |
| Poly(ZHis$_4$-NAPGly) (**1**) | 20% Chol/DPPC liposomes | 6.6 | 4.7 |
| ZHis-hexylamide (**3**) | 20% Chol/DPPC liposomes | 4.1 | 37.5 |
| Poly(ZHis$_4$-NAPGly) (**1**) | 1% PS/DPPC liposomes | 7.2 | 11.8 |
| ZHis-hexylamide (**3**) | 1% PS/DPPC liposomes | 4.1 | 46.1 |

Table 4

Relative rate constant for hydrolysis of *p*-nitrophenyl esters at 35 °C in the presence of liposomes

Values of $k_{cat}$ (in l mol$^{-1}$ min$^{-1}$) for hydrolysis of PNPA and PNPP by imidazole in 50% dioxane/50% buffer mixture were 5.6 and 2.2, respectively.

| Catalyst | Solvent | $k_{cat}^{rel}$ | |
|---|---|---|---|
| | | PNPA | PNPP |
| Imidazole | 50% dioxane | 1.0 | 1.0 |
| ZHis-hexylamide (3) | 50% dioxane | 0.45 | 0.50 |
| Poly(ZHis$_4$-NAPGly) (1) | DPPC liposomes | 3.6 | 1.4 |
| Poly(C$_{12}$ZHis$_3$-NAPGly) (2) | DPPC liposomes | 4.1 | 6.8 |
| ZHis-hexylamide (3) | DPPC liposomes | 11.7 | 117 |
| Poly(ZHis$_4$-NAPGly) (1) | DMPC liposomes | 6.1 | 1.7 |
| Poly(C$_{12}$ZHis$_3$-NAPGly) (2) | DMPC liposomes | 12.5 | 15.4 |
| ZHis-hexylamide (3) | DMPC liposomes | 13.0 | 129 |
| Poly(ZHis$_4$-NAPGly) (1) | 20% Chol/DPPC liposomes | 5.1 | 4.3 |
| ZHis-hexylamide (3) | 20% Chol/DPPC liposomes | 2.0 | 18.3 |

The absolute rate constant for hydrolysis of PNPA by imidazole or ZHis-hexylamide (3) in 50% dioxane/50% buffer mixture was greater than that of PNPP, indicating steric hindrance due to the long acyl chain having exerted an effect on the bimolecular reaction. Therefore, no specific interaction is considered to occur between the substrate and catalyst in solution.

$k_{cat}^{rel}$ for hydrolysis of PNPA by poly(ZHis$_4$-NAPGly) (1) amounted to 11.7, compared to only 2.0 for PNPP. Since poly(ZHis$_4$-NAPGly) (1) is adsorbed to the membrane surface, it hydrolyzes PNPA efficiently which also becomes adsorbed to the membrane surface. On the other hand, the hydrophobic polypeptide catalyst, poly(C$_{12}$ZHis$_3$-NAPGly) (2), hydrolyzed PNPA and PNPP more efficiently than did poly(ZHis$_4$-NAPGly) (1). In particular, $k_{cat}^{rel}$ for the hydrolysis of PNPP by poly(C$_{12}$ZHis$_3$-NAPGly) (2) was 10-fold greater than that for poly(ZHis$_4$-NAPGly) (1). It is therefore concluded that hydrolysis of the hydrophobic substrate PNPP in the presence of liposomes is facilitated by a more hydrophobic polypeptide catalyst that is more strongly bound to the lipid membrane. This trend is even more pronounced for hydrolysis of PNPP catalyzed by ZHis-hexylamide (3). Since ZHis-hexylamide (3) is incorporated into the lipid membrane to a deeper level, PNPP hydrolysis became much more rapid than PNPA hydrolysis.

The hydrolytic reactions of PNPA and PNPP catalyzed by these polypeptides at 35 °C were slower in gel-state DPPC membranes than in liquid-crystalline DMPC membranes (table 4). Lateral diffusion of bound substrate and flexibility of the catalytic site of the polypeptides should decrease in gel-state membranes, leading to low $k_{cat}^{rel}$ values in such membranes.

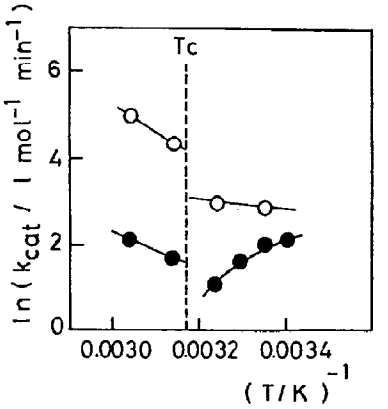


Fig. 1. Arrhenius plot of $k_{cat}$ for hydrolysis catalyzed by poly(ZHis$_4$-NAPGly) (1) in the presence of DPPC liposomes. $T_c$, phase-transition temperature of DPPC liposomes. $k_{cat}$ of: (○) PNPA, (●) PNPP.

The absolute rate constant for PNPA hydrolysis by poly(ZHis$_4$-NAPGly) (1) below the phase-transition temperature of DPPC liposomes (35 °C) had a lower value as compared to that above $T_c$ (45 °C). In contrast, the corresponding values for PNPP do not differ very much. The temperature dependence of $k_{cat}$ for catalysis of poly(ZHis$_4$-NAPGly) (1) in the presence of DPPC liposomes was investigated in detail, the results being shown in fig. 1. Above the phase-transition temperature, the $k_{cat}$ values for both substrates increased with rising temperature. On the other hand, below $T_c$, $k_{cat}$ for PNPA showed a slight decrease with falling temperature, while $k_{cat}$ for PNPP increased. The latter observation appears to be unusual. It has been reported that the properties of membranes close to phase-transition temperature can be interpreted on the basis of the coexistence of both gel and liquid-crystalline phases in the membranes [19,20]. Furthermore, phase separation into domains comprising pure lipid and others containing high concentrations of solute has been demonstrated to occur in membranes below $T_c$ [21]. Therefore, increasing local concentrations of substrate and polypeptide below the phase-transition temperature must be the reason for the increase in $k_{cat}$ for PNPP observed at low temperatures. Conversely, $k_{cat}$ for PNPA showed no increase, probably because the binding of PNPA to the membrane surface is so loose that the phase separation induced by decreasing temperature has no influence.

The effect of mixing cholesterol in DPPC membranes on hydrolytic activities of polypeptide catalysts is difficult to explain. Cholesterol addition strongly suppressed the hydrolysis of PNPA and PNPP catalyzed by ZHis-hexylamide (3) at either 45 or 35 °C, whereas hydrolysis by poly(ZHis$_4$-NAPGly) (1) was enhanced except in the case of PNPA at 45 °C. Generally speaking, the effect of cholesterol addition on hydrolysis by ZHis-hexylamide (3) might be the reverse of that by the polypeptide catalyst (1). The marked decrease in hydrolytic activity of ZHis-hexylamide (3) induced on addition of cholesterol above $T_c$ should be due to the decrease in membrane fluidity, which consequently hinders bimolecular collisions in the membrane phase. At the same time, ZHis-hexylamide (3) might be squeezed out of the lipid membrane or move toward the surface region of the lipid membrane on cholesterol addition, since the degree of penetration of molecules into lipid membranes is a function of the density of packing of lipid molecules in the membranes. It has been shown that pancreatic phospholipase $A_2$ loses hydrolytic activity in tightly packed membranes, due to being inaccessible to membranes [22]. Furthermore, the nucleophilicity of the imidazolyl group will be strongly diminished when undergoing interaction with the polar head groups of lipid molecules. For example, Murakami et al. [9,23] have reported that the nucleophilicity of nucleophiles is dependent on the location in comicelles as a result of varying electrostatic interactions. In contrast, since the binding of polypeptide catalyst (1) to DPPC membranes was enhanced by cholesterol addition, the corresponding increase in rate constant can be explained in terms of the rise in local concentrations of the catalytic sites of polypeptide catalyst (1) in DPPC membranes. The

Table 5

Substrate selectivity of polymer catalysts ($k_{cat}^{rel}$ for PNPA/$k_{cat}^{rel}$ for PNPP) in the presence of different liposomes

| Catalyst | 35 °C | | | 45 °C | | |
|---|---|---|---|---|---|---|
| | 20% Chol/DPPC | DPPC | DMPC | 1% PS/DPPC | 20% Chol/DPPC | DPPC |
| Poly(ZHis$_4$-NAPGly) (1) | 1.2 PNPA | 2.6 PNPA | 3.6 PNPA | 0.6 PNPP | 1.4 PNPA | 5.9 PNPA |
| Poly($C_{12}$ZHis$_3$-NAPGly) (2) | – | 0.6 PNPP | 0.8 PNPP | – | – | 1.4 PNPA |
| ZHis-hexylamide (3) | 0.1 PNPP | 0.1 PNPP | 0.1 PNPP | 0.09 PNPP | 0.1 PNPP | 0.07 PNPP |

latter effect should overwhelm the counter effect of decreasing membrane fluidity.

PS addition to DPPC membrane increased the rate constant for hydrolysis of PNPP by poly-($ZHis_4$-NAPGly) (**1**) at 45 °C. This observation can also be explained on the basis of greater local concentrations of polymer catalyst (**1**) in the membranes due to electrostatic attractions. This is consistent with the explanation for the higher hydrolytic activity of poly($C_{12}ZHis_3$-NAPGly) (**2**) being due to stronger hydrophobic interactions with lipid membranes.

Table 5 summarizes data on the substrate selectivity of the present peptide catalysts in the presence of liposomes. $k_{cat}^{rel}$(PNPA)/$k_{cat}^{rel}$(PNPP) ratios above unity present PNPA specificity, those below unity corresponding to PNPP specificity. Concomitant with the membrane affinity of the peptide catalysts increasing in the order, (**1**) < (**2**) < (**3**), PNPP selectivity increases. Furthermore, it should be noted that addition of cholesterol or PS to DPPC membranes reduced the selectivity for PNPA in catalysis by poly($ZHis_4$-NAPGly) (**1**). In conformity with the above, the substrate selectivity and catalytic efficiency of the polypeptide catalysts can be controlled by changing the nature of the membrane environment.

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